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There were errors in the manuscript originally published online. In the "Conclusion" of the Abstract and in the titles of Tables 1 and 2, the exposure levels for arsenic should have been given as "µg/L" instead of "µg/µL" or "ppm." The errors have been corrected here.

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Evaluation of the Association between Arsenic and Diabetes: A National Toxicology Program Workshop Review

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Contributors

Dana Loomis served as chair and Elizabeth Maull served as rapporteur for the arsenic breakout group. Other members of the arsenic breakout group included: Habibul Ahsan, Glinda Cooper, Joshua Edwards, Matthew P. Longnecker, Ana Navas-Acien, Jingbo Pi, Ellen Silbergeld, Miroslav Styblo, and Chin-Hsiao Tseng. Kristina Thayer was primary author of the background literature review document prepared prior to the workshop.

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Abbreviations

aP2 – fatty acid-binding protein

As(III) – arsenite; trivalent arsenic

As(III) oxide – arsenic trioxide; As2O3

As(V) – arsenate; pentavalent arsenic

As(V) oxide – arsenic pentoxide; As2O5

CC – case control

C/EBPα – CCAAT/enhancer binding protein (C/EBP) alpha

CEI – cumulative exposure index

CS – cross sectional

DMA(III) – dimethylarsinite

DMA(III) oxide – dimethylarsine oxide

DMA(V) – dimethylarsinate

FBG – fasting blood glucose

GLUT4 - glucose transporter type 4

GSIS – glucose stimulated insulin secretion

HbA1c – hemoglobin A1c; glycosylated hemoglobin

HEALS – Health Effects of Arsenic Longitudinal Study

HIF1a – hypoxia inducible factor, alpha

HO1 – heme-oxygenase 1

HOMA-IR - homeostasis model assessment of insulin resistance

ipGTT – ip glucose tolerance test

IRS - insulin receptor substrate

IUF1 – insulin upstream factor 1 (also known as PDX1)

KLF5 – Kruppel like factor 5

LOEC – lowest observed effect concentration

LOEL – lowest observed effect level

MAPKAP-K2 – mitogen-activated protein kinase-activated protein kinase 2

MMA(III) – monomethylarsonite

MMA(V) – monomethylarsonate

MAs(III) oxide – methylarsine oxide

NAC – n-acetyl cysteine

NOEC – no observed effect concentration

NOEL – no observed effect level

Nrf2 – transcription factor NF-E2-related factor 2

OGTT – oral glucose tolerance test

PDX1 – pancreatic and duodenal homeobox 1 (also known as IUF1)

PPARg – peroxisome proliferator-activated receptor gamma

T2D – Type 2 diabetes

ABSTRACT

Background: Diabetes affects an estimated 346 million people globally. Total deaths from diabetes are projected to increase > 50% in the next decade. Understanding the role of environmental chemicals in the development or progression of diabetes is an emerging issue in environmental health. In 2011, the National Toxicology Program (NTP) organized a workshop to assess the literature for evidence of associations between certain chemicals, including inorganic arsenic, and diabetes and/or obesity to help develop a focused research agenda. This report is derived from discussions at that workshop.

Objectives: Our objective was to assess the consistency, strength/weaknesses, and biological plausibility of findings in the scientific literature regarding arsenic and diabetes, and to identify data gaps and areas for future evaluation/research. The extent of the existing literature was insufficient to consider obesity as an outcome.

Data Sources, Extraction, and Synthesis: Studies related to arsenic and diabetes or obesity were identified through PubMed and supplemented with relevant studies identified by reviewing the reference lists in the primary literature or review articles.

Conclusions: Existing human data provide "limited" to "sufficient" support for an association between arsenic and diabetes in populations with relatively high exposure levels (≥ 150 μg arsenic/L in drinking water). The evidence is "insufficient" to conclude that arsenic is associated with diabetes in lower exposure (<150 g arsenic/L drinking water), although recent studies with better measures of outcome and exposure support an association. The animal literature as a whole was inconclusive; however, studies using better measures of diabetes-relevant endpoints support a link between arsenic and diabetes.

INTRODUCTION

Diabetes, both Type 1 and Type 2 (T2D), is a major threat to public health in the United States and abroad (CDC 2011; Danaei et al. 2011; WHO 2011). Based on data from the 2005-2008 National Health and Nutrition Examination Survey (NHANES), approximately 25.6 million, or 11.3%, of all people in the United States aged ≥20 years have diagnosed or undiagnosed diabetes, resulting in estimated direct medical costs and indirect costs (disability, work loss, premature death) of $174 billion in 2007 alone (CDC 2011). Another 35% of people ≥20 years of age are pre-diabetic (American Diabetes Association 2011; Knowler et al. 2002). Recently, diabetes is being diagnosed in individuals earlier in life (NIDDK 2011). Although approximately 70% of T2D is attributed to being overweight or obese (Eyre et al. 2004), 30% of T2D cases are not attributable to obesity. Given the number of people impacted by T2D-- 346 million worldwide (WHO 2011)-- and its long term consequences in terms of morbidity, mortality, and economic costs, there is considerable interest in understanding the contribution of "non-traditional" risk factors to the diabetes epidemic, including environmental chemicals.

Research addressing the role of environmental chemicals in diabetes manifestation has rapidly expanded. The February 2011 Diabetes Strategic Plan from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK 2011) acknowledged the need to understand the role of environmental exposures as part of future research and prevention strategies. To help develop such a research strategy, the National Toxicology Program organized a state-of-the-science workshop in January 2011 entitled "Role of Environmental Chemicals in the Development of Diabetes and Obesity" (National Toxicology Program 2011b). The objective of this workshop

was to assess the literature for evidence of associations between diabetes and/or obesity with chemicals including arsenic, persistent organic pollutants, maternal smoking during pregnancy, bisphenol A, phthalates and organotins, and non-persistent pesticides (Thayer et al. 2012). This report is derived from discussions on arsenic that occurred at the workshop.

The arsenic evaluation focused on diabetes only, as studies have not assessed obesity as a primary health outcome. Our review focused on (1) the consistency, strength/weaknesses, and biological plausibility of findings, (2) identification of the most useful and relevant endpoints in experimental animals and mechanistic studies, and (3) identification of data gaps and areas for future evaluation/research.

IDENTIFICATION OF RELEVANT STUDIES

A PubMed search strategy, first conducted on August 24, 2009 and then run weekly until December 15, 2010, was developed to identify human, animal, and mechanistic studies (including in vitro assays) on arsenic exposures related to diabetes and obesity using MeSH-based and keyword strategies (see Supplemental Material, page 2 for search terms). A total of 108 publications were identified from the search and 38 of these presented original data concerning both arsenic exposure and diabetes (or diabetes-related endpoints and/or mechanisms) and were considered relevant (see Supplemental Material, Figure S1). An additional 38 studies were identified during the course of the initial primary literature review and discussions with workshop participants, including two studies that had been submitted, but not yet accepted for publication (Del Razo et al. 2011; Paul et al. 2011), for a total of 76 studies

considered as the final primary literature. Two of these studies included more than one type of data, human and animal (Wang et al. 2009) or animal and in vitro (Yen et al. 2007).

A goal of the review was to assess the scientific literature using the descriptors "sufficient", "limited", or "insufficient" to classify existing evidence, with NTP definitions utilized for the NTP Report on Carcinogens as a framework for "sufficient" and "limited" (National Toxicology Program 2011a). "Sufficient" evidence for human studies indicates a causal relationship between exposure to the agent, substance, or mixture and an outcome based on evidence of a dose-response and other characteristics such as consistency and coherence among different studies, adequate control for other covariates, biological plausibility, and adequate identification of sources of potential bias. "Limited" evidence indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded. The term "insufficient" is used when there is low confidence in the body of evidence to reach a conclusion on the association between exposure to a substance and health outcome(s); or no data are available.

Epidemiological studies were classified as: (1) occupational; (2) population-based studies in areas with relatively high environmental arsenic exposure (≥ 150 µg/L in drinking water); (3) population-based studies in areas with lower arsenic exposure (< 150 µg/L in drinking water) excluding NHANES (National Health and Nutrition Examination Survey) studies; and (4) NHANES studies. The cutpoints used for drinking water arsenic were selected to distinguish between "high exposure" studies in areas with unusually high exposures via drinking water (e.g., in areas of Taiwan and Bangladesh) and "low-to-moderate" exposure studies.

EPIDEMIOLOGICAL STUDIES

The first epidemiological studies reporting associations between arsenic and diabetes were published in mid-1990s. These early studies were conducted in populations exposed to high levels of arsenic in drinking water in Taiwan and Bangladesh, or occupational studies of copper smelter and glass workers in the United States and Europe exposed to dust and particulates as distinct from water. Previous reviews of studies published before 2008 concluded that arsenic exposure was most consistently associated with diabetes in areas of Taiwan and Bangladesh with high arsenic contamination of drinking water in the past, while results from occupational studies and studies of populations with "low to moderate" arsenic levels in drinking water were inconsistent (Chen et al. 2007; European Food Safety Authority 2009; Longnecker and Daniels 2001; Navas-Acien et al. 2006; Tseng et al. 2002). More than 10 new epidemiological studies of arsenic exposure and diabetes have been published since 2007.

Detailed descriptions of all of the epidemiological studies considered for the review can be found in the technical literature review document prepared for the NTP workshop [see "Draft Literature Review Documents" at http://ntp.niehs.nih.gov/go/36433 (National Toxicology Program 2011b)]. Eight occupational studies also were considered as part of the review (see Supplemental Material, Table S1) but are not considered further in this report because of concerns about diabetes assessment, exposure misclassification, and limited power. Most of the occupational studies ascertained diabetes based on death certificates, which are well known to have low sensitivity and specificity for diabetes (Cheng et al. 2008). In addition, arsenic exposure was determined based on job title, and with one exception (Lubin et al. 2000), the sample size or

number of individuals with diabetes was small. This assessment of the occupational studies is consistent with other reviews of arsenic (Longnecker and Daniels 2001; Navas-Acien et al. 2006).

Environmental exposure settings

Of the 27 eligible non-occupational publications that met our inclusion criteria, 9 were classified as "high exposure" (Table 1), 15 were classified as non-NHANES studies with "low to moderate exposure" (Table 2), 1 was classified as both low and high exposure (Chen et al. 2010), and 4 were based on analysis of NHANES data (Table 2). Two high-exposure studies used a prospective design (Tseng et al. 2000a, 2000b), and the rest were cross-sectional (n=12, excluding the NHANES studies), case-control (n=5), or retrospective (n=4). Three studies did not report risk estimates for diabetes, but compared the levels of arsenic in diabetics and non-diabetics (Afridi et al. 2008; Kolachi et al. 2010; Serdar et al. 2009).

Diabetes ascertainment differed among studies. Four studies used death certificates to ascertain diabetes (Lewis et al. 1999; Meliker et al. 2007; Tollestrup et al. 2003; Tsai et al. 1999) and three others used exclusively self-reported history of diabetes (Afridi et al. 2008; Chen et al. 2010; Zierold et al. 2004). Two studies used diagnosis of diabetes but did not report the basis of diabetes diagnosis (Ruiz-Navarro et al. 1998; Ward and Pim 1984). Seven studies, generally those conducted more recently, incorporated diagnostic indicators such as fasting glucose or oral glucose tolerance test (OGTT) (Coronado-Gonzalez et al. 2007; Del Razo et al. 2011; Ettinger et al. 2009; Kolachi et al. 2010; Rahman et al. 1998; Tseng et al. 2000b; Wang et al. 2007). Two other studies reported risk estimates for metabolic syndrome (Wang et al. 2007) and impaired

glucose tolerance (Ettinger et al. 2009) rather than diabetes. Many of the studies were conducted in Bangladesh [n = 4; (Chen et al. 2010; Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999)] or Taiwan [n = 5; (Lai et al. 1994; Tsai et al. 1999; Tseng et al. 2000b; Wang et al. 2007; Wang et al. 2003)]. Other countries included the United States (Ettinger et al. 2009; Lewis et al. 1999; Meliker et al. 2007; Navas-Acien et al. 2008, 2009a; Steinmaus et al. 2009a, b; Tollestrup et al. 2003; Zierold et al. 2004), Mexico (Coronado-Gonzalez et al. 2007; Del Razo et al. 2011), Pakistan (Afridi et al. 2008; Kolachi et al. 2010), Turkey (Serdar et al. 2009), Spain (Ruiz-Navarro et al. 1998), China (Wang et al. 2009) and the United Kingdom (Ward and Pim 1984).

Measures of exposure are highly variable between these studies, ranging from area-wide exposure estimates based on measurement of arsenic from drinking water sources to individual-level exposure estimates based on detailed water consumption history, work history, or actual biomarkers of exposure. These variations in study design constitute irreducible sources of heterogeneity and present interpretive challenges in evaluating the results observed in this collection of studies. Specifically, exposure was assessed by arsenic concentrations in drinking water within a geographic area (Del Razo et al. 2011; Meliker et al. 2007; Zierold et al. 2004), as cumulative exposure index based on residence time x average drinking water level (Chen et al. 2010; Lai et al. 1994; Lewis et al. 1999; Rahman et al. 1999; Tseng et al. 2000b), residence time in arsenicosis-endemic region (Tollestrup et al. 2003; Tsai et al. 1999; Wang et al. 2003), presence or absence of arsenicosis or keratosis as a surrogate for long-term exposure to arsenic (Nabi et al. 2005; Rahman et al. 1998), or by biomarkers including blood/plasma arsenic levels (Ettinger et al. 2009; Serdar et al. 2009; Ward and Pim 1984), and arsenic concentration in urine (Coronado-Gonzalez et al. 2007; Navas-Acien et al. 2008, 2009a; Ruiz-Navarro et al. 1998;

Steinmaus et al. 2009a, b; Wang et al. 2009), or hair (Afridi et al. 2008; Kolachi et al. 2010; Wang et al. 2007). Three studies did not report risk estimates but compared the levels of arsenic in diabetics and non-diabetes. Afridi et al. (2008) measured higher levels of arsenic in the hair, blood, and urine of 196 diabetics participating in a study that included a total of 434 men from Hyderabad, Pakistan. Higher arsenic urine, blood, and hair levels were also found in diabetics compared to non-diabetics in another study conducted in Pakistan by Kolachi et al. (2010). Levels of hair arsenic were significantly higher in a group of 76 new mothers with insulin-dependent diabetes compared to a group of 68 non-diabetic mothers, although it should be noted that hair is not considered the preferred matrix for arsenic (National Research Council 1999). Serdar et al. (2009) did not detect any statistically significant differences in plasma arsenic in diabetes cases (n = 31, mean ± SD = 1.22 ± 0.57 µg/L) compared to controls [n = 22; mean (range) = 0.86 (0.64 - 1.59 µg/L)] in a study based in Turkey, although this study may have been underpowered to detect differences.

Environmental exposure, high arsenic areas (≥ 150 µg/L drinking water)

Figure 1 summarizes the high arsenic environmental exposure studies from Bangladesh (Chen et al. 2010; Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999) and Taiwan (Lai et al. 1994; Tsai et al. 1999; Tseng et al. 2000a, 2000b; Wang et al. 2003). There is "limited" to "sufficient" evidence for an association between arsenic and diabetes in populations from high arsenic areas, primarily occurring in Bangladesh or Taiwan. Support for an association was strongest in studies where arsenic drinking water levels > 500 ug/L (Lai et al. 1994; Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999; Tsai et al. 1999; Tseng et al. 2000b; Wang et al. 2003). Eight of the nine studies conducted in Taiwan or Bangladesh reported positive associations between

arsenic and diabetes (Figure 1) (Lai et al. 1994; Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999; Tsai et al. 1999; Tseng et al. 2000b; Wang et al. 2003). The only prospective study within this group also reported a positive association [adjusted relative risk (RR) = 2.1 (95% CI: 1.1 – 4.2)] for development of diabetes over a four-year follow-up period among individuals with ≥ 17 compared with < 17 mg/L-yrs cumulative arsenic exposure (Tseng et al. 2000b). Those studies relying on clinically accepted measures of disease (e.g., fasting blood glucose, OGGT) (Tseng et al. 2000a, 2000b; Rahman et al. 1998; Lai et al. 1994) reported risk estimates ranging from 2.1 (RR; 95% CI: 1.1 – 4.2) to 10.05 (adjOR; 95% CI: 1.3 – 77.9). It is worth noting that some of the studies might not be completely independent if they are surveying the same population, and perhaps the same individuals. Of the studies conducted in Taiwan, several (Lai et al. 1994; Tsai et al. 1999; Tseng et al. 2000b; Wang et al. 2003) derived their study populations from the Southwestern Blackfoot or arseniasis-endemic region of Taiwan. Furthermore, several papers specifically include the village of Pu-Tai (Lai et al. 1994; Tseng et al. 2000a, 2000b). Data presented in the Tseng publications (Tseng et al. 2000a, 2000b) represent a follow-up to the Lai et al. (1994) study and therefore likely included many of the same participants. Studies conducted in Bangladesh have focused on the same geographical area for their exposed populations: Dhaka, Rajshahu, and Khulna Divisions (Chen et al. 2010;Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999). While none of the Bangladesh studies indicated that they are follow-up activities related to previous studies, participants may have overlapped.

In contrast to the relative strength and consistency of results in many of the high exposure studies, the most recent and largest study in Bangladesh did not find any significant associations between urinary arsenic or time-weighted water arsenic and self-reported diabetes, glucosuria or

hemoglobin A1c (HbA1c) levels in a population-based cross sectional study of 11,319 Bangladeshi men and women participating in the Health Effects of Arsenic Longitudinal Study (HEALS) (Chen et al. 2010). Diagnosis of diabetes was based on self-report of physician diagnosis prior to baseline, glucosuria (excluding 90 individuals who were taking medications for diabetes), or, in a smaller subset of 2,100 participants, HbA1c. Although the Chen et al. (2010) cohort is large, statistical power was limited by the small number of diabetes cases (241 of 11,078, or ~ 2% of the total cohort reported a diagnosis of diabetes prior to baseline, including 45 diabetes cases in the highest quintile category for TWA arsenic). Nonetheless, while a number of explanations for the findings of Chen et al. (2010) exist, no definitive conclusions could be drawn regarding aspects of the study design or population (e.g., nutritional status, obesity, genetic differences), or exposure history (i.e., the relatively short duration of exposure for some study participants compared with the experiences of individuals in the arsenic-contaminated areas of Taiwan) that could explain the difference between this and the other studies.

Environmental exposure, low-to-moderate arsenic areas

Excluding the NHANE studies, 12 of the 15 identified epidemiologic studies reported risk estimates related to diabetes, glycemic control, or metabolic syndrome in populations under conditions of low-to-moderate arsenic exposure from drinking water (< 150 µg/L drinking water) (Table 2). Two studies (Lewis et al. 1999; Meliker et al. 2007) evaluated SMRs for each gender separately. The highest categories of drinking water exposure in these studies were lower than the arsenic exposed population studies in Bangladesh and Taiwan. Overall, the current literature provides “insufficient” evidence to conclude that arsenic is associated with diabetes at these levels of exposure. Recent studies with better measures of outcome (fasting blood glucose

levels or OGTT) reported more consistent associations between arsenic and diabetes (Coronado-Gonzalez et al. 2007; Del Razo et al. 2011) or impaired glucose tolerance (Ettinger 2009) within this range of exposure. Some of the differences among the studies may be due to variation in sample sizes, and differences in study populations and methods used to classify diabetes (e.g., death certificates versus self-report or blood glucose) or estimate arsenic exposure (e.g., urine levels versus drinking water surveys).

Four publications based on analyses of data from NHANES cohorts, which are representative of the U.S. population and generally include participants with low to moderate exposure, were consider in our review (Navas-Acien et al. 2008, 2009a; Steinmaus et al. 2009a, 2009b). However, these studies should not be considered independent results as the main focus of several of the publications was to compare the methodological strategies used to assess the association between urine arsenic and diabetes. In brief, differences in interpretation of the association between arsenic and diabetes can be reached based on different methodological approaches used to account for organic arsenic due to seafood consumption and whether or not to include urinary creatinine as an adjustment factor in the statistical model. Results of two of the NHANES analyses supported an association between arsenic exposure and diabetes (Navas-Acien et al. 2008, 2009a), but results based on two alternative analyses did not (Steinmaus et al. 2009a, 2009b). Differences in methodological approaches used to characterize arsenic exposure in these studies are discussed in more detail below under “Urinary arsenic.”

Determining Exposure and Internal Dose in Studies of Arsenic

Arsenic concentrations in drinking water

Measurement of total arsenic in drinking water supplies is often used to assess arsenic exposure, but this approach is not appropriate for research questions pertaining to individual exposures, including research concerning the effects of individual variation in arsenic metabolism on internal dose. Individual-level information on the magnitude, duration, and timing of exposure is critical, especially for estimating cumulative exposure. One alternative to has been to combine historical measurements of arsenic concentrations in drinking water with self-reported residential and water use histories. This approach usually requires an assumption that arsenic concentrations in drinking water are stable over time, and that study subjects do not consume water from other sources. Support for these assumptions has been found in several study populations (Navas-Acien et al. 2009b; Ryan et al. 2000).

Arsenic levels in blood, nails and hair

The literature review revealed a number of arsenic exposure biomarkers in need of further characterization and validation. Whole blood and plasma are emerging exposure matrices that reflect a shorter half-life (about one-hr) compared to arsenic levels in urine (four days) (National Research Council 1999). Hair and nail arsenic levels are non-invasive measures that reflect average arsenic levels for exposures that occurred several months (hair) to over a year (nails) before sampling (Orloff et al. 2009). Moreover, arsenic levels in nails generally reflect exposure to inorganic arsenic, and seem to be less affected by seafood arsenicals (see below). While

sometimes useful, hair is not a recommended exposure matrix for arsenic (National Research Council 1999). One limitation of measuring arsenic in hair and nails is that arsenic speciation is difficult to conduct. Also, the time period of exposure captured by hair and nail measurements depends on the specific segments collected and analysed. Other target tissues (e.g., urothelial cells) and buccal and saliva samples have also been suggested (Bartolotta et al. 2011; Hernandez-Zavala et al. 2008; Lew et al. 2010). While these emerging biomarkers deserve additional attention, a more expanded knowledge of toxicokinetic data and information on correlations with existing biomarkers and intake doses is needed before they are adopted for use in research.

Urinary Arsenic

One of the most commonly used measures of arsenic exposure is urine. However, measurements of total urinary arsenic will not distinguish between inorganic and organic forms of arsenic unless a speciated analysis is conducted. Distinguishing between the inorganic and organic forms of arsenic is important because the inorganic forms are generally accepted as being of greater toxicological concern than the organic forms [Agency for Toxic Substances and Disease Registry (ATSDR) 2007; Vahter and Concha 2001]. The metabolism of inorganic arsenic is complex and results in a number of metabolites, including some that are chemically unstable. Inorganic arsenic occurs in two oxidation states: arsenite [As(III)] and arsenate [As(V)], where the Roman numeral refers to the oxidation state. In the process of forming more water soluble molecules, inorganic arsenic goes through alternating reduction and methylation reactions and fluctuates between oxidation states of III (regarded as more toxic) and V (less toxic) (ATSDR 2007; Vahter and Concha 2001). It is worth noting that the general characterization of oxidation state III as

less toxic than V is primarily based on acute toxicity studies and this issue has not been adequately assessed in long-term toxicological studies.

In any case, total urinary arsenic reflects the number of arsenic ions generated from all arsenic species in the urine, including inorganic arsenic [As(III), As(V)], the tri- and pentavalent methylated metabolites of inorganic arsenic [monomethylarsonite, MMA(III); dimethylarsinite, DMA(III); monomethylarsonate, MMA(V), and dimethylarsinate, DMA(V)], and the less toxic organic arsenic compounds commonly associated with dietary exposures, particularly in seafood (mainly arsenobetaine, arsenosugars, and arsenolipids) (Caldwell et al. 2009; Navas-Acien et al. 2009b) (Figure 1; also, see Supplemental Materials, Table S2 for detailed information on common forms of arsenic). Since it is currently assumed that both the inorganic forms of arsenic and their methylated metabolites may be associated with diabetes and other health risks, speciation analysis, including specification of the arsenic oxidation state, is recommended. Studies that do include a speciated analysis often do not include an oxidative state analysis to distinguish between tri- and pentavalent metabolites of inorganic arsenic. In particular, there is a need to improve the ability to measure methylated trivalent species because they are regarded as more toxic (ATSDR 2007; Vahter and Concha 2001) and concentrations may be underestimated unless the appropriate speciation analysis is conducted. Although technically challenging and not typically done, it is possible to conduct analyses of these metabolites at the point of collection.

Accounting for arsenic of seafood origin: Most human biomonitoring studies report levels of total arsenic, which includes inorganic and organic arsenic compounds and their metabolites. Depending upon location and diet of the population being studied, fish and other seafood can be

a significant source of exposure to specific organic forms of arsenic such as arsenobetaine, arsenosugars, and arsenolipids (Figure 1). Although they have not been evaluated as risk factors for diabetes-related endpoints, these complex organic arsenic compounds are generally accepted as less toxic than either inorganic arsenic or their methylated metabolites (ATSDR 2007; Vahter and Concha 2001). Inorganic arsenic as well as methylated forms in oxidation state III are highly reactive, with a high affinity for sulfhydryl groups (Vahter and Concha 2001). Therefore, failure to distinguish organoarsenicals from inorganic arsenic and metabolites of inorganic arsenic in urine may result in misclassification of exposure to the most toxicologically relevant forms of arsenic, which may lead to mischaracterization of the association between urine arsenic and diabetes. This is less of a concern when study participants are exposed to higher levels of arsenic from drinking water or proximity to an industrial or mining site with arsenic contamination, as it is reasonable to assume that urinary arsenic primarily reflects exposure to inorganic arsenic in these populations. However, in studies of the general population, such as NHANES, a larger portion of urinary arsenic may represent organic arsenic, mostly due to seafood consumption (Longnecker 2009; Navas-Acien et al. 2009; Steinmaus et al. 2009a).

How to best adjust for organic arsenicals of seafood origin is a controversial topic (see Supplemental Materials for a detailed discussion). Inorganic forms, arsenite and arsenate, are metabolized to their methylated forms, MMA and DMA, and eliminated in the urine. However, while DMA is the major metabolite of inorganic arsenic, it is also a metabolite of the organic arsenicals, arsenosugars and arsenolipids, and therefore reflects both exposures to inorganic and organic forms of arsenic of seafood origin (Figure 1). Three published strategies have been used to address this issue using NHANES data: (1) statistically adjusting models used to estimate the

association between total urinary arsenic and diabetes for markers of seafood intake, such as levels of urinary arsenobetaine and blood mercury (Navas-Acien et al. 2008); (2) restricting the analysis to participants with very low or non-detectable levels of arsenobetaine (Navas-Acien et al. 2009a); and (3) subtracting any organic arsenicals above detection limits (i.e., arsenobetaine and arsenocholine) from the total urinary arsenic measurement (Steinmaus et al. 2009a). These strategies lead to different conclusions regarding the association between inorganic arsenic and diabetes in NHANES, with the first two approaches resulting in statistically significant associations (Navas-Acien et al. 2011; Navas-Acien et al. 2009a), while the third suggested no association (Steinmaus et al. 2009a). Subtracting arsenobetaine from total arsenic does not account for exposure misclassification due to the presence of other seafood arsenicals and their metabolites in urine, which are included in total urinary arsenic measurements but cannot be specifically accounted for because they were not measured separately in the NHANES samples. Statistical adjustment for arsenobetaine and restriction to participants with low levels of arsenobetaine control for all seafood arsenic species, not only for arsenobetaine, and have shown consistent results in several studies (Navas-Acien et al. 2011; Navas-Acien et al. 2009a). However, statistical adjustment may not completely eliminate bias because it mixes the effects of relevant and irrelevant exposures, and exclusion of seafood consumers from analysis may lead to selection bias in populations where seafood consumption is common. The lack of consistency of findings based on the different analytical approaches described above warrants caution in interpreting results from NHANES studies and highlights the importance of having good analytical methods to distinguish inorganic arsenic and its methylated metabolites from organic arsenicals of seafood origins.

Accounting for urine dilution: Typically, epidemiology studies that quantify exposure based on spot urine measures for arsenic or other non-persistent chemicals include adjustments for urine creatinine to account for variation in urine dilution. This may be accomplished through normalizing arsenic levels for creatinine as the exposure metric (i.e., μg As/g urinary creatinine) or adjusting by using urine arsenic as the measure of exposure (μg As/L urine), but then including creatinine as a separate independent variable in the multiple regression analyses. Of the two approaches, the latter approach is recommended (Barr et al. 2005) because urinary creatinine concentrations are influenced by age, sex, health status, race/ethnicity, body mass index, fat-free mass, and time of day of collection and can therefore vary widely across individuals (Barr et al. 2005; Boeniger et al. 1993; Mahalingaiah et al. 2008). However, this strategy may not be appropriate for metals or other chemicals that compromise kidney function.

The decision on how, or whether, to adjust for urinary creatinine concentration is more complicated when the health effect under investigation can impact creatinine levels, as is the case with diabetes (Greenland 2003). People with diabetes tend to have lower urinary concentrations of creatinine, in part because muscle mass is reduced as a consequence of diabetes, which results in reduced creatinine excretion (Park et al. 2009). Diabetes also leads to increased glomerular filtration and increased water intake, which can cause urine to be more dilute, resulting in lower urinary creatinine concentrations (Jerums et al. 2010). Both of the physiological processes may lead to biased assessments on the association between urinary arsenic and diabetes, although it is not possible to predict the direction of the overall bias with confidence, i.e., systematic bias towards or away from identifying a positive association. The reasons for this are discussed in more detail in the literature review document prepared for the

2011 workshop (National Toxicology Program 2011b). The situation is further complicated because arsenic exposure has also been associated with increased urine creatinine in people living in an arsenic endemic area of Bangladesh (Nermell et al. 2008) or participating in the HEALS study described above (Ahsan H, personal communication). Thus, if diabetes and arsenic affect creatinine production, as well as urine dilution, then adjustment for creatinine may introduce bias rather than controlling measurement error induced by urine dilution (Greenland 2003). Relative risk estimates for associations between arsenic and diabetes based on creatinine-adjusted urine are quantitatively higher than estimates based on urine arsenic levels that are not adjusted for creatinine (Chen et al. 2010; Steinmaus et al. 2009b). However, given the issues discussed above it may not be possible to fully understand the potential bias with respect to clarifying the association between arsenic and diabetes. While specific gravity has been suggested as an alternative method to normalize urinary arsenic for differences in urine dilution because it appears to be less affected than creatinine by age, gender, and body size (Mahalingaiah et al. 2008; Nermell et al. 2008), its use is not recommended in studies of diabetes because it is well established that specific gravity is not an accurate method if albumin or glucose is present in the urine (Chadha et al. 2001; Voinescu et al. 2002). One approach to address concerns about creatinine-adjustment is to report both raw and adjusted values. Prospective evidence, that is, measuring arsenic and creatinine at baseline and then diabetes development over the follow-up, remains the best strategy to eliminate potential bias related to the impact of diabetes in urine creatinine concentrations, i.e., prior to any potential renal or metabolic effect of the disease in urine creatinine.

Emerging issues related to arsenic exposure

At present, there is very little exposure or toxicity information of other types of arsenicals. Roxarsone, an arsenic-based drug fed to chicken, turkeys, and pigs for growth promotion, feed efficiency, and improved pigmentation, may be a source of dietary exposure to inorganic arsenic (Silbergeld and Nachman 2008; U.S. Food & Drug Administration 2011). Thioarsenical metabolites in urine are emerging forms of concern but are difficult to measure and their interpretation is at present unclear (Naranmandura et al. 2010; Pinyayev et al. 2011). The significance of the gut microbiome in understanding arsenic toxicity is another new issue in the field. Available data suggest the impact of microbiome metabolism of arsenic prior to absorption into the human body may be important in terms of interpreting observed differences in patterns of arsenic metabolites in addition to differences in metabolic pathways within human organs (Proctor 2011; Sun et al. 2012; Van de Wiele et al. 2010).

EXPERIMENTAL ANIMAL STUDIES

Over 20 animal studies published since 1979 were identified for this review, primarily conducted in rats or mice (Figure 2). The existing studies are highly diverse, with considerable variation in the duration of treatment (one day to two years), routes of administration, and in doses used in the studies. The most common routes of administration were oral, predominantly through drinking water or diet, or intraperitoneal injections. Other, less common forms of administration were gavage, oral capsules, or subcutaneous injection. Most of the studies treated animals with As(III) or arsenic trioxide, but other arsenicals have also been studied (Aguilar et al. 1997; Arnold et al. 2003; Hill et al. 2009; Paul et al. 2008). The studies also vary in experimental design and model systems used to assess endpoints relevant to diabetes as a health effect, ranging from urinary glucose in fasted animals (Pal and Chatterjee 2005), to blood glucose in non-fasted animals (Mitchell et al.

2000), to glucose tolerance test (Cobo and Castineira 1997; Ghafghazi et al. 1980; Hill et al. 2009; Paul et al. 2008; Paul et al. 2007b; Paul et al. 2011; Wang et al. 2009). Glucose was a commonly reported endpoint but findings were inconsistent across studies which may stem from differences in biological compartment assessed (urine, serum, plasma, whole blood) and fasting status of the animal (fasted, non-fasted, fasting status not reported) in addition to the differences in experimental design noted above related to arsenical tested, species, route of administration, and dose levels (Aguilar et al. 1997; Arnold et al. 2003; Biswas et al. 2000; Boquist et al. 1988; Ghafghazi et al. 1980; Hill et al. 2009; Izquierdo-Vega et al. 2006; Judd 1979; Mitchell et al. 2000; Pal and Chatterjee 2004a, 2004b, 2005; Paul et al. 2008; Paul et al. 2007b; Paul et al. 2011; Wang et al. 2009). Although the literature as a whole was judged inconclusive, findings from recent studies that were designed to focus more specifically on diabetes-relevant endpoints appear, at least qualitatively, to support a link between arsenic exposure and diabetes. Supportive findings include impaired glucose tolerance in studies with mice (Boquist et al. 1988; Hill et al. 2009; Paul et al. 2007b; Paul et al. 2011; Yen et al. 2007), or rats (Cobo and Castineira 1997; Ghafghazi et al. 1980; Izquierdo-Vega et al. 2006; Singh and Rana 2009; Wang et al. 2009). Measures of insulin regulation , i.e., HOMA-IR, insulin sensitivity (Paul et al. 2011), as well as pancreatic effects, including indicators of oxidative stress, degenerative changes in β-cells, and pancreatitis (Arnold et al. 2003; Boquist et al. 1988; Izquierdo-Vega et al. 2006; Mukherjee et al. 2006; Yen et al. 2007), have also been reported to be affected. Results from several animal studies suggest that co-treatment with methyl donors or antioxidants (e.g., folic acid, vitamin B_{12}, methionine, N-acetyl cysteine) may attenuate the effects of arsenic toxicity, including reductions in the degree of arsenic-induced pancreatic toxicity (Mukherjee et al. 2006) and arsenic-induced hyperglycemia (Pal and Chatterjee 2004a, 2004b, 2005). Although not directly assessing the

potential diabetogenic effects of arsenic, a study by Reichl et al. (1990) reported that co-treatment with glucose increased the survival rate in NMRI mice treated with a dose of As(III) oxide that resulted in 100% mortality when administered without the glucose (12.9 mg/kg by sc injection).

These studies suggest that animal models can be relevant to understand effects of arsenic on glycemic control depending on experimental design. Mice may be less susceptible than humans to arsenic toxicity, partly due to faster metabolism and clearance of arsenic resulting in lower internal dose of inorganic arsenic species (Paul et al. 2008; Paul et al. 2007b). Rats, unlike mice or humans, sequester arsenic (specifically DMA) in erythrocytes (Lu et al. 2007; Lu et al. 2004; Lu et al. 2008). It is unclear how this binding affects target organ dose of inorganic arsenic and rats are generally not recommended as a model for assessing arsenic metabolism or toxicity (National Research Council 1999).

MECHANISMS

A number of in vitro studies implicate several pathways by which arsenic can influence pancreatic β-cell function and insulin sensitivity, including oxidative stress, glucose uptake and transport, gluconeogenesis, adipocyte differentiation, and Ca^{2+} signalling (reviewed in Diaz-Villasenor et al. 2007; Druwe and Vaillancourt 2010; Tseng 2004; see also Figure 3). Several of these pathways are discussed in more detail below, but in general the studies fall into the following categories: (1) that utilize high concentrations of arsenic (≥ 1 mM) to examine stress response in various cell types, although the concentrations used limit interpretation because they are not considered physiologically relevant, resulting in cytotoxicity; (2) studies that test lower

concentrations (< 100 μM) of arsenic that report inhibition of insulin signaling and insulin-dependent glucose uptake by adipocytes or myotubes (Paul et al. 2007b; Walton et al. 2004; Yen et al. 2010); and (3) studies in insulinoma cell lines or isolated pancreatic islets that suggest that the mechanisms by which arsenic affects β-cells to inhibit insulin expression and/or secretion are concentration dependent (Díaz-Villaseñor et al. 2006; Pi et al. 2007; Díaz-Villaseñor et al 2008; Fu et al. 2010). At relatively low concentrations (in the sub-μM range) certain adaptive cellular responses to arsenic-induced oxidative stress [i.e., induction of antioxidant enzymes and reduced reactive oxygen species (ROS)] may result in an impairment of glucose-stimulated insulin secretion (Pi et al. 2007; Fu et al. 2010). High concentrations result in irreversible damage (including oxidative damage) to β-cells followed by apoptosis or necrosis (Macfarlane et al. 1999; Macfarlane et al. 1997; Ortsater et al. 2002).

Influence of inorganic arsenic on glucose-stimulated insulin secretion in pancreatic β-cells

Chronic oxidative stress leading to oxidative damage has long been implicated in β-cell dysfunction in diabetes. Oxidative stress is also implicated in many aspects of arsenic toxicity and a recent in vitro study suggests that Nrf2-mediated antioxidant response may influence arsenite-induced impairment of glucose-stimulated insulin secretion in β-cells at low concentrations of arsenite (Fu et al. 2010). The transcription factor Nrf2 is a key cellular component that defends cells against toxicities of oxidants and electrophiles by regulating both constitutive and inducible expression of many antioxidant/detoxification enzymes (Fu et al. 2010; Gomez-Rubio et al. 2011; He and Ma 2010; Sergeev and Carpenter 2011). While antioxidants are generally considered protective for cells, this same Nrf2-driven induction of endogenous antioxidant enzymes meant to maintain intracellular redox homeostasis and limit

oxidative damage may also have a negative impact on insulin secretion by diminishing the availability of ROS, such as hydrogen peroxide (H_2O_2). Reactive oxygen species signals produced during glucose metabolism are becoming recognized as intracellular regulators of glucose-stimulated insulin secretion acting to increase insulin secretion (Leloup et al. 2009; Pi et al. 2007; Pi et al. 2010).

Thus, Nrf2-mediated antioxidant response appears to play paradoxical roles in β-cell function by (1) blunting glucose-triggered 'ROS signalling' and thus resulting in reduced glucose-stimulated insulin secretion and (2) protecting β-cells from oxidative damage and subsequent apoptosis/necrosis (Fu et al. 2010). Chronic exposure to inorganic arsenic and the production of its methylated trivalent metabolites have been linked to oxidative stress; however, at the levels generally expected in low-to-moderate human exposures, they are not likely to reach cytotoxic concentrations sufficient to cause irreversible damage, although at these levels they may activate Nrf2. Therefore, premise (1) above is potentially more relevant to β-cell dysfunction in the context of low-level environmental arsenic exposure, whereas premise (2) might be associated with β-cell damage and failure induced by high doses of arsenic.

Influence of trivalent arsenicals on glucose uptake in adipocytes and skeletal muscle cells

Type 2 diabetes is characterized by disruptions in whole-body glucose homeostasis due to insulin resistance and impaired glucose utilization by peripheral tissues, including skeletal muscle and adipose tissue. Results of tissue culture studies suggest that arsenite and/or its methylated trivalent metabolites cause insulin resistance in adipocytes by inhibiting insulin signalling and insulin-activated glucose uptake. Arsenite can also interfere with the formation of insulin

sensitive adipocytes and myotubes by inhibiting adipogenic and myogenic differentiation (Salazard et al. 2004; Trouba et al. 2000; Walton et al. 2004; Yen et al. 2010).

Arsenite and its metabolites interact with a number of elements involved in insulin signalling, including insulin receptor substrate (IRS), phosphatidylinositol-3 kinase (PI3K), AKT, phosphoinositide-dependent kinase (PDK), and protein kinase C (PKC). AKT belongs to a class of enzymes important in regulating glucose metabolism, cell proliferation, apoptosis, transcription and cell migration (Paul et al. 2007a; Walton et al. 2004). Insulin stimulates glucose uptake by binding to the insulin receptor and activating the IRS-1, IRS-2, PI3K, PDK, AKT and/or PKC-ζ, PKC-λ signalling pathway(s) (Choi and Kim 2010; Standaert et al. 1999). Activation of PKC-ζ and PKC-λ stimulates Ras-related protein (RAB4A) activity, the association of RAB4A with kinesin-like protein KIF3B, and the interaction of KIF3B with microtubules. This process is essential for recruitment of glucose transporter type 4 (GLUT4) to the cytoplasmatic membrane and for insulin-dependent glucose uptake (Imamura et al. 2003; Lee et al. 2010). Sub-cytotoxic concentrations in the μM range of arsenite and its methylated trivalent metabolites, MMA(III) and DMA(III), inhibit insulin-stimulated glucose uptake in cultured adipocytes by interfering with the phosphorylation of AKT-dependent mobilization of GLUT4. Arsenite and MMA(III) inhibit PDK-catalyzed phosphorylation of AKT in the insulin signalling cascade; DMA(III) inhibits GLUT4 translocation by interfering with the signalling step(s) downstream from AKT (Paul et al. 2007a; Walton et al. 2004). Adaptive antioxidant response associated with prolonged exposure to relatively low concentrations of arsenite in the 1 to 2 μM range have also been associated with suppression of insulin-stimulated AKT phosphorylation and glucose uptake in 3T3-L1 adipocytes to cause an insulin resistant phenotype (Xue et al.

2011).

Insulin resistance is a hallmark of diabetes and the role of adipocytes in mediating insulin resistance is an active area of research. A number of studies have assessed the impact of arsenic on adipocytes. Arsenite inhibits and reverses differentiation of adipocytes by disruption of expression of the genes involved in adipogenesis (Wauson et al. 2002). Expression of both peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) is required for phenotypic differentiation of adipocytes and arsenite inhibits expression of both of these transcription factors. Arsenite disrupts the interaction between PPARγ and its coactivator retinoid X receptor alpha (RXRα). Arsenic trioxide also inhibits AKT binding to PPARγ (Wang et al. 2005). Inhibition of these transcription factors reduces expression of PPARγ and C/EBPα target genes: adipocyte fatty acid binding protein (A-FABP), which is involved in preadipocyte differentiation, and p21, a protein whose expression is tightly regulated during adipogenesis (Wang et al. 2005; Wauson et al. 2002). Inhibition of p21 leads to activation of preadipocyte proliferation, thereby inhibiting adipocyte differentiation (Wang et al. 2005).

Myogenesis is associated with the development of the insulin-responsive glucose transport system and there are indications that arsenite may have similar effects on myogenic differentiation; however, this has not been studied to the same extent as effects on adipocytes. Pathways mediating muscle differentiation include insulin-dependent activation of AKT/mTOR/p70 S6 kinase1/MEF2C/MYOD/MYOG signalling (Conejo et al. 2002; Xu and Wu 2000). Low concentrations (20 nM) of arsenite have been shown to delay the differentiation of muscle cells from myoblasts to myotubes by repressing the transcription factor myogenin

(Steffens et al. 2010). Arsenite also significantly decreases the phosphorylation of AKT and its downstream targets, mTOR and p70 S6 kinase1 proteins, during myogenic differentiation (Yen et al. 2010). Inhibition of AKT by arsenite was also demonstrated in muscle cells (Yen et al. 2010), and may lead to a reduction in glucose uptake in this tissue (Diaz-Villasenor et al. 2007).

CONCLUSIONS AND RESEARCH NEEDS

Overall, data from human studies included in this review support an association between inorganic arsenic and diabetes in populations with arsenic drinking water levels > 500 µg/L (Lai et al. 1994; Nabi et al. 2005; Rahman et al. 1998; Rahman et al. 1999; Tsai et al. 1999; Tseng et al. 2000b; Wang et al. 2003), but the currently available evidence was considered "insufficient" to conclude that arsenic is associated with diabetes in individuals with low-to-moderate exposure (< 150 µg/L in drinking water). Stronger evidence of associations at lower levels of exposure based on some recent studies with better measures of outcome and exposure support the need for further research in populations with low to moderate exposure levels. Weaknesses noted in the epidemiological literature review included a lack of prospective studies, use of death certificates or self-reported diagnosis for ascertainment of diabetes, and ecological methods of exposure assessment. Because of these limitations, the evidence of effects at high arsenic exposure levels ranged from "limited" to "sufficient", but did not reach the threshold for a "sufficient" classification.

Research needs identified as a result of this literature review are summarized in Table 3. Prospective studies in areas of lower exposure (e.g., parts of North America other than arsenic-

endemic regions) with individual measurements of exposure prior to disease incidence are needed. However, utilization of existing cohorts (such as the Strong Health Study), nested case-control designs, and follow-up of cross-sectional populations such as NHANES is also recommended. Additional consideration of the results from the recent HEALS study in Bangladesh (Chen et al. 2010), which do not align with findings from other studies in areas of moderate to high exposure, would also be helpful to better understand factors that influence the generalizability of associations reported based on other study populations. Research on interactions between arsenic exposure and factors such as BMI, diet, levels of physical activity, co-exposures including metals that occur with arsenic, duration of exposure, and timing of exposure (i.e., the importance of early life or prenatal exposures) may help address this issue. In addition, future studies should include consideration of gene-environment interactions, including studies of polymorphisms in genes related to arsenic metabolism and diabetes susceptibility.

Given its well-established role as a risk factor for diabetes the impact of obesity as a potential modifying factor needs to be better addressed, especially in countries such as the United States and Mexico where overweight and obesity are epidemics (WHO 2012). Average body mass index (BMI) in Bangladesh and Taiwan, where the association between arsenic exposure and diabetes was stronger, is much lower than in the United States and Mexico. For example, approximately ~80% of study participants in the HEALS study in Bangladesh had a BMI of <22 (Chen et al. 2010) whereas 68% of study participants included in the analysis of NHANES 2008 had a BMI of ≥25 (Navas-Acien et al. 2008). In the Mexico studies, 34% to 50% of participants had a BMI >30 (Coronado-Gonzalez et al. 2007; Del Razo et al. 2011). Information on BMI was not presented in most of the studies conducted in Taiwan except for Tseng et al. (2000b), where

the average BMI was 24.5 kg/m^2; although as a population, the prevalence of overweight/obesity is higher in Taiwan compared to Bangladesh and lower compared to the United States (Huang 2008; WHO 2012). Many of the recent studies considered BMI as a potential confounding factor (Chen et al. 2010; Coronado-Gonzalez et al. 2007; Del Razo et al. 2011; Ettinger et al. 2009; Kim and Lee 2011; Lai et al. 1994; Navas-Acien et al. 2008, 2009a; Rahman et al. 1999; Steinmaus et al. 2009a, 2009b; Tseng et al. 2000b) but the issue of obesity as an effect modifier or potential intermediate on a causal pathway between arsenic and diabetes has not been well-explored in the existing literature.

The experimental animal literature as a whole was judged inconclusive, but findings from recent studies that focus on diabetes-relevant endpoints appear consistent with those human studies that support a link between arsenic exposure and diabetes. Moreover, the animal studies implicate several pathways by which arsenic may influence pancreatic β-cell function and insulin sensitivity, and suggest novel biomarkers for understanding pathways of response to arsenic in human populations. However, animal studies need to be designed to be relevant to human exposures in terms of internal dose. Use of specific inbred strains susceptible to diabetes and metabolic syndrome may also be informative. Application of systems toxicology approaches within the framework utilized by the NIEHS and others in studying relevance of the "toxosome" to the "diabetome" may be innovative and stimulate new information on key signalling pathways that connect arsenic to diabetes.

Overall, animal studies need to be designed to specifically evaluate the influence of arsenic on the development of diabetes, using modern methods and well characterized endpoints for

diabetes. Blood glucose levels, both fasting and fed, as well as insulin levels were identified as appropriate endpoints for animal studies. The influence of adiposity on the development of arsenic-induced diabetes could be explored more fully in animal models by quantitating fat-mass and distribution in both white and brown adipose tissues.

Improved methodologies are needed for more accurate environmental exposure assessments as well as for internal dosemetrics and biologically based measurements that integrate all and differentiate among exposures, metabolites, and toxicities. Some of the newer proposed biomarkers (toe and fingernails, saliva, buccal cells) need to be further characterized in terms of their relationships to external exposures and validated.

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Table 1. Association between arsenic and diabetes in areas of relatively high exposure (≥150 µg/L drinking water)

Refrence and Study Design	Subjects	Diabetes Diagnosis	Main Finding[a,b]	Exposure[c]	Factors Considered in Analysis
(Chen et al. 2010) cross-sectional	Bangladesh (Araihazar) HEALS, n=11,319 ♂♀	self-report prior to baseline	1.11 (95% CI: 0.73, 1.69) adjOR	176.2-864 (Q5) vs 0.1-8 (Q1) µg As/L drinking water, CEI *cohort: 0.1 – 864 µg As/L*	age, gender, BMI, smoking status, and educational attainment
(Lai et al. 1994) cross-sectional	Taiwan (Southern) As endemic region, n=891 ♂♀	self-report, oral glucose tolerance test, treatment history	10.05 (95% CI: 1.3, 77.9) adjOR	≥ 15 vs 0 ppm-y drinking water, CEI *cohort: 780 (700 – 930) µg As/L; median (range) concentrations in artesian wells**	age, sex, BMI, physical activity
(Nabi et al. 2005)[d] case-control	Bangladesh (Chapainowabganj) arsenicosis cases, n=235 ♂♀	glucose, blood	2.95 (95% CI: 0.954, 9.279) OR	218.1 vs 11.3 (avg) µg As/L drinking water *cohort: 218.1 (3 – 875) µg As/L; average (range)*	unadjusted
(Rahman et al. 1998)[e] cross-sectional	Bangladesh (Dhaka) keratosis cases, n=1,107 ♂♀	self-report, oral glucose tolerance test, glucosuria	5.2 (95% CI: 2.5, 10.5) adjPR	keratosis vs non-keratosis *cohort: <10 – 2100 µg As/L*	age
(Rahman et al. 1999)[e] cross-sectional	Bangladesh (multi-site) w/skin lesions, n=430 ♂♀	glucosuria	2.9 (95% CI: 1.6, 5.2) adjPR	>10 vs <1 mg-y As/L drinking water, CEI *cohort: <500 to >1,000 µg As/L drinking water*	age, sex, BMI
(Tsai et al. 1999)[d] retrospective	Taiwan (Chiayi County) Blackfoot region, n=19,536 deaths ♂♀	death certificate	1.46 (95% CI: 1.28, 1.67) SMR	Blackfoot endemic region vs national reference *cohort: 780 (250 – 1140) µg As/L; median (range)*	age, sex
(Tseng et al. 2000a; Tseng et al. 2000b) prospective	Taiwan (southwestern) agricultural and aquacultural regions, n=446 ♂♀	fasting blood glucose, oral glucose tolerance test	2.1 (95% CI: 1.1, 4.2) RR	≥ 17 vs < 17 mg/L-y As (drinking. water, CEI) *cohort: 700 – 930 µg As/L; range of median concentration in artesian wells*	age, sex, BMI
(Wang et al. 2003)[f] cross-sectional	Taiwan (southwestern) As endemic region, n=706,314 ♂♀	insurance claims	2.69 (95% CI: 2.65, 2.73) adjOR	endemic vs non-endemic region *cohort: 780 (350 – 1140) µg As/L; median (range)**	age, sex

Table 1. Association between arsenic and diabetes in areas of relatively high exposure (≥150 µg/L drinking water)

adjOR – adjusted odds ratio; adjPR – adjusted prevalence ratio; As- arsenic; avg – average; BMI – body mass index; CEI – cumulative exposure index; CI – Confidence Interval; HEALS – Health Effects of Arsenic Longitudinal Study; km – kilometre; mg-y – milligram year; mi – miles; N – number; OR – odds ratio; ppm-y – parts per million year; Q – quintile; RR – relative risk; SMR – standardized mortality ratio; U.S. – United States; WA – Washington

[a]Identification of main findings was based on the following strategy: For studies that did not report a significant association between arsenic exposure and a health outcome at any exposure level, the main summary finding was based on the highest exposure group compared to the referent group (e.g., 4th quartile versus 1st quartile). When a study reported a significant association between arsenic exposure and a health outcome the main finding was based on lowest exposure group where a statistically significant association was observed (e.g., 3rd quartile versus 1st quartile).

[b]Unless specified, relative risk estimates are crude estimates.

[c]Median or average and range of As concentration in drinking water for the cohort is included when reported

[d]Calculated by entering data presented in publication into OpenEpi software (Dean et al. 2011).

[e]Although the arsenic water concentrations are expressed units of mg/L, the value is supposed to represent the "Approximate time-weighted mean arsenic exposure levels were calculated over the lifetime of each subject as 2y-(a,- CjyZjdj, where a} is the number of years a well with arsenic concentration cy- was used, assuming that the current levels of arsenic in the well water were also representative of the past."

[f]There appears to be an error on the number of people included in the "non-endemic" area category based on the n's provided in Table 1.

*Arsenic drinking water concentrations taken from other publications based on same populations.

Table 2. Association between arsenic and diabetes-related measures in areas of relatively low to moderate exposures (<150 µg/L drinking water) and NHANES

Refrence and Study Design	Subjects	Diabetes Diagnosis	Main Finding[a,b]	Exposure[c]	Factors Considered in Analysis
(Afridi et al. 2008)[d] cross-sectional	Pakistan (Hyderabad), n=225 ♂ (non-smokers) and n=209 ♂ (smokers)	self-report	↑ urinary As in non-smoking diabetics	non-smokers: 5.59 (diabetics) vs 4.7 (non-diabetics) µg As/L, average (urine) smokers:7.27 (diabetics) vs 5.41 (non-diabetics) µg As/L *cohort: drinking water concentrations not reported*	unadjusted
(Chen et al. 2010) cross-sectional	Bangladesh (Araihazar) HEALS, n=11,319 ♂♀	self-report prior to baseline	1.24 (95% CI: 0.82, 1.87) adjOR	41-92 (Q3) vs 0.1-8 (Q1) µg As/L drinking water, CEI *cohort: 0.1 – 864 µg As/L*	age, gender, BMI, smoking status, and educational attainment; (similar results obtained when model only adjusted for age, gender, and BMI)
(Coronado-Gonzalez et al. 2007) case-control	Mexico (Coahuila) As endemic region, n=400 ♂♀	fasting blood glucose, treatment history	2.84 (95% CI: 1.64, 4.92) adjOR	>104 (T3) vs < 63.5 (T1) µg As/g Cr (urine) *cohort: 20–400 µg As/L drinking water reported in other studies of the region*	age, sex, hypertension, family history, obesity, and serum lipids
(Del Razo et al. 2011) cross-sectional	Mexico (Zimapan & Lagunera) As endemic region, n=258 ♂♀	fasting blood glucose	1.13 (95% CI: 1.05, 1.22) adjOR per 10 µg As/L ↑	*cohort: 42.9 average (3-215 range) µg As/L (current drinking water)*	age, sex, obesity and hypertension
(Ettinger et al. 2009) cross-sectional	U.S. (Tar Creek, OK) n=456 pregnant ♀	impaired glucose tolerance (oral glucose tolerance test)	2.79 (95% CI: 1.13, 6.87) adjOR	2-24 (Q4) vs 0.2-0.9 (Q1) µg As/L (blood) *cohort: reported from other studies that at least 25% of samples in region have >10 µg As/L drinking water*	age, pre-pregnancy BMI, ethnicity/race, Medicaid use, married or living with partner
(Kolachi et al. 2010) case-control	Pakistan (Hyderabad) diabetes, n=144 ♀	IDDM (fasting blood glucose, oral glucose tolerance test)	↑ urine As in diabetics	4.13 (diabetics) vs 1.48 (non-diabetics) µg As/L, average (urine) *cohort: drinking water concentrations not reported*	unadjusted
(Lewis et al. 1999) retrospective	U.S. (7 communities in Millard County, UT), n=961 ♀ deaths; n=1,242 ♂ deaths	death certificate	♀: 1.23 (95% CI: 0.86, 1.71) SMR ♂: 0.79 (95% CI: 0.48, 1.22) SMR	Millard vs state *cohort: 14 – 166 µg (3.5 – 620) µg As/L, range of median well water concentrations between 1976-1997 (overall range)*	sex, race

Table 2. Association between arsenic and diabetes-related measures in areas of relatively low to moderate exposures (<150 µg/L drinking water) and NHANES

Refrence and Study Design	Subjects	Diabetes Diagnosis	Main Finding[a,b]	Exposure[c]	Factors Considered in Analysis
(Meliker et al. 2007) retrospective	U.S. (6 counties in southeastern MI) n=41,282 ♂ deaths; n=38,722 ♀ deaths	death certificate	♂: 1.28 (95% CI: 1.18, 1.37) SMR ♀: 1.27 (95% CI: 1.19, 1.35) SMR	6 counties vs state µg As/L (drinking water) *cohort: 7.58 (1.27 – 11.98) µg As/L, population weighted median across 6 counties (range)*	sex, race
(Ruiz-Navarro et al. 1998)[f] case-control	Spain (Motril) hospital patients, n=87 ♂♀	not reported	0.87 (95% CI: 0.5, 1.53) RR	75th vs 25th percentile µg As/L (urine) *cohort: drinking water concentrations not reported*	unadjusted
(Serdar et al. 2009) cross-sectional	Turkey (Ankara) n=87 diabetes clinic patients	treatment history	↔ plasma As in diabetics versus controls	1.22 (diabetics) vs 0.86 (non-diabetics) µg As/L (plasma) *cohort: drinking water concentrations not reported*	unadjusted
(Tollestrup et al. 2003)[f] retrospective	U.S. (Ruston, WA) lived near smelter as children, n=1,074 deaths ♂♀	death certificate	1.6 (95% CI: 0.36, 7.16) RR	Resisdence time within 1.6 km (1 mi): ≥ 10 years vs <1 year *cohort: drinking water concentrations not reported*	unadjusted
(Wang et al. 2007) cross-sectional	Taiwan (central) industrial region, n=660 ♂♀	metabolic syndrome (fasting blood glucose, triglycerides, HDL, blood pressure, BMI)	2.35 (95% CI: 1.02, 5.43) adjOR	"high" vs "low" µg As/g hair *cohort: 2002-2005 ground water concentrations for area ranged from ~6 to ~15* µg As/L	age, sex, occupation, lifestyle factors (alcohol, betel nut chewing, smoking, groundwater use)
(Wang et al. 2009)[e] cross-sectional	China (Xinjiang region) As endemic region, n=235 ♂♀	hospital records, exam	1.098 (95% CI: 0.98, 1.231) RR	21-272 (range) vs 16-38 (range) µg As/L (drinking water) *cohort: 16-272* µg As/L *drinking water*	unadjusted
(Ward and Pim 1984)[f] case-control	U.K. (Oxford, England) diabetes clinic patients, n=117 ♂♀	not reported	1.09 (95% CI: 0.79, 1.49) RR	75th vs 25th percentile µg As/mL (plasma) *cohort: drinking water concentrations not reported*	unadjusted
(Zierold et al. 2004)[g] cross-sectional	U.S. (WI) well-water testing program, n=1,185 ♂♀	self-report	1.02 (95% CI: 0.49, 2.15) adjOR	>10 vs <2 µg As/L (well-water) *cohort: 2 (0 – 2,389) µg As/L;median (range)*	age, sex, BMI, smoking

Table 2. Association between arsenic and diabetes-related measures in areas of relatively low to moderate exposures (<150 µg/L drinking water) and NHANES

Refrence and Study Design	Subjects	Diabetes Diagnosis	Main Finding[a,b]	Exposure[c]	Factors Considered in Analysis
(Navas-Acien et al. 2008) cross-sectional	U.S. (NHANES 2003-04) ≥ 20y, n=788 ♂♀	fasting blood glucose, self-report, medication	3.58 (95% CI: 1.18, 10.83) adjOR	18 (≥80th) vs 3.5 (≤20th percentile) µg As/L (urine)	sex, age, race, and urine creatinine, education, BMI, serum cotinine level, hypertension medication, urine arsenobetaine, blood mercury levels
(Navas-Acien et al. 2009a) cross-sectional	U.S. (NHANES 2003-06) ≥20y, n=1,279 ♂♀ with arsenobetaine < LOD	fasting blood glucose, self-report, medication	2.60 (95% CI: 1.12, 6.03) adjOR	7.4 (80th) vs 1.6 (20th) µg As/L (urine)	sex, age, race, and urine creatinine, education, BMI, serum cotinine level, hypertension medication, blood mercury levels
(Steinmaus et al. 2009a) cross-sectional	U.S. (NHANES 2003-04) ≥20y, n=795 ♂♀	fasting blood glucose, self-report, medication	1.15 (95% CI: 0.53, 2.50) adjOR	12 (≥80th) vs 2.7 (≤20th) µg As/L (urine, not adjusted for creatinine) [urine As = total As-(arsenobetaine+arsenocholine)]	sex, age, ethnicity, education, BMI, serum cotinine, urine creatinine, current use of hypertension medications
(Steinmaus et al. 2009b) cross-sectional	U.S. (NHANES 2003-06) ≥20y, n= ~1,280 ♂♀ with arsenobetaine < LOD	fasting blood glucose, self-report, medication	1.03 (95% CI: 0.38, 2.80) adjOR	≥80th vs ≤ 20th percentile µg As/L (urine, not adjusted for creatinine)	sex, age, race, BMI

adjOR – adjusted odds ratio; adjPR – adjusted prevalence ratio; As- arsenic; avg – average; AsB – arsenobetaine; AsCh – arsenocholine; BMI – body mass index; CEI – cumulative exposure index; CI – Confidence Interval; Cr – creatinine; HDL – high density lipoproteins; IDDM – insulin dependent diabetes mellitus; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; LOD – level of detection; MD – Maryland; MI – Michigan; N – number; NHANES – National Health and Nutrition Examination Survey; NIDDM – non-insulin dependent diabetes mellitus; NR – not reported; OK – Oklahoma; Q – quintile; Qr – quartile; RR – relative risk; SMR – standardized mortality ratio; T – tertile; U.K. – United Kingdom; U.S. – United States; UT – Utah; WA – Washington

[a]Identification of main findings was based on the following strategy: For studies that did not report a significant association between arsenic exposure and a health outcome at any exposure level, the main summary finding was based on the highest exposure group compared to the referent group (e.g., 4th quartile versus 1st quartile). When a study reported a significant association between arsenic exposure and a health outcome the main finding was based on lowest exposure group where a statistically significant association was observed (e.g., 3rd quartile versus 1st quartile).

[b] Unless specified, relative risk estimates are crude estimates.

[c]Median or average and range of As concentration in drinking water included, when privided in the primary literature.

[d]The standard deviations presented in the study may be SEMs.

[e]Calculated by entering data presented in publication into OpenEpi software (Dean et al. 2011).

[f]Relative risk and 95% confidence interval as estimated by Navas-Acien et al. (2006).

[g]Number of cases were not reported in original study but were reported in Navas-Acien et al (2006)..

Table 3. Research Needs

Epidemiology	• Prospective studies with incident cases for diabetes, especially at lower exposure ranges – Consider utilizing existing cohorts, nested case-control design, and follow-up of cross-sectional populations • Impact of early-life exposures • Impact of arsenic metabolism • Impact of diet, BMI, and physical activity • Genetic susceptibility related to both response to arsenic and diabetes • Epigenetic research related to mechanisms • Investigate potential increased risk for Type 1 diabetes and gestational diabetes
Exposure	• Exposure data on other arsenicals, i.e., thioarsenicals, roxarsone • Method development for urinary DMA(III) and MMA(III) and measurement of arsenic metabolites in blood • Co-exposure between arsenic and other chemicals including metals • Cost effect strategies for analysis and markers of seafood arsenic • Better characterization of other biomarkers of exposure, i.e., toe and fingernails (non-invasive and reflects long-term exposure), saliva, buccal cells, target tissues • Validate spot urine findings with 24-hr urine samples for a sample of the study population
Animal and In Vitro	• Identify animal models appropriate for arsenic induced-diabetes – Need to consider internal dose • Epigenetic research that includes an emphasis on developmental effects • Assess low-concentration effects in vitro • Mechanisms of glucose homeostasis in other tissues (in vitro)

FIGURE LEGENDS

Figure 1. Arsenic exposure and metabolism in the human body: from source to urine [modified from Navas-Acien et al. (2009a)].

*Arsenic species measured in NHANES (Caldwell et al. 2009). Two other organic forms of arsenic considered to be minor contributors to arsenic in seafood were also measured in NHANES but only detected in a small number of urine samples, arsenocholine (1.8%) and trimethylarsine oxide (0.3%). The predominant urinary metabolite of arsenocholine in rats, mice and rabbits is arsenobetaine (Marafante et al. 1984).

Figure 2. Animal studies of arsenic and endpoints related to glucose homeostasis

Abbreviations: As(III) - arsenite; As(III) oxide – arsenic trioxide; As(V) - arsenate; As(V) oxide – arsenic pentoxide; d – day; GD – gestation day; HFD – high fat diet; HOMA-IR - homeostasis model assessment of insulin resistance; hr – hour; ip – intraperitoneal; ipGTT – ip glucose tolerance test; LFD – low fat diet; MAs(III) oxide – methylarsine oxide; min – minutes; MMA - monomethylarsonate; NR – not reported; OGTT – oral glucose tolerance test; wk – week; y – year

[ ] = bracketed information indicates that the dose was converted to mg/kg from a different dose unit presented in the publication; use of brackets can also indicated that experimental details were not explicitly stated in the paper but could be reasonably inferred.

‡Notes on Arnold et al. (2003) rat findings: Effects on blood glucose in rats were only observed at one year of age, not at study completion at two years of age; the occurrence of pancreatitis was not statistically different in the high dose group compared to controls, but did display a

significant dose-related trend ($p > 0.001$) in both male and female rats.

Figure 3. In vitro studies related to arsenic and diabetes

Abbreviation: aP2 – fatty acid-binding protein; As2O3 – arsenic trioxide; As2O5 – arsenic pentoxide; As(III) – arsenite; As(V) – arsenate; Ca – calcium; C/EBPα – CCAAT/enhancer binding protein (C/EBP alpha); DMA(III) oxide – dimethylarsine oxide; DMA(V) – dimethylarsinate; GSIS – glucose stimulated insulin secretion; HIF1a – hypoxia inducible factor, alpha; HO1 – heme oxygenase 1; IUF1 – insulin upstream factor 1 (also known as PDX1); KLF5 – Kruppel like factor 5; LOEC – lowest observed effect concentration; MAPKAP-K2 – mitogen-activated protein kinase-activated protein kinase 2; MAs(III) oxide – methylarsine oxide; MAs(V) – monosodium methylarsonate; mRNA – messenger RNA; NAC – N-acetyl cysteine; NOEC – no observed effect concentration; Nrf2 – transcription factor NF-E2-related factor 2; PDX1 – pancreatic and duodenal homeobox 1 (also known as IUF1); PhAsO – oxophenylarsine; PPARg – peroxisome proliferator-activated receptor gamma; ROS – reactive oxygen species

Δ = cytotoxicity reported at specified concentration level

Figure 1. Arsenic exposure and metabolism in the human body: from source to urine

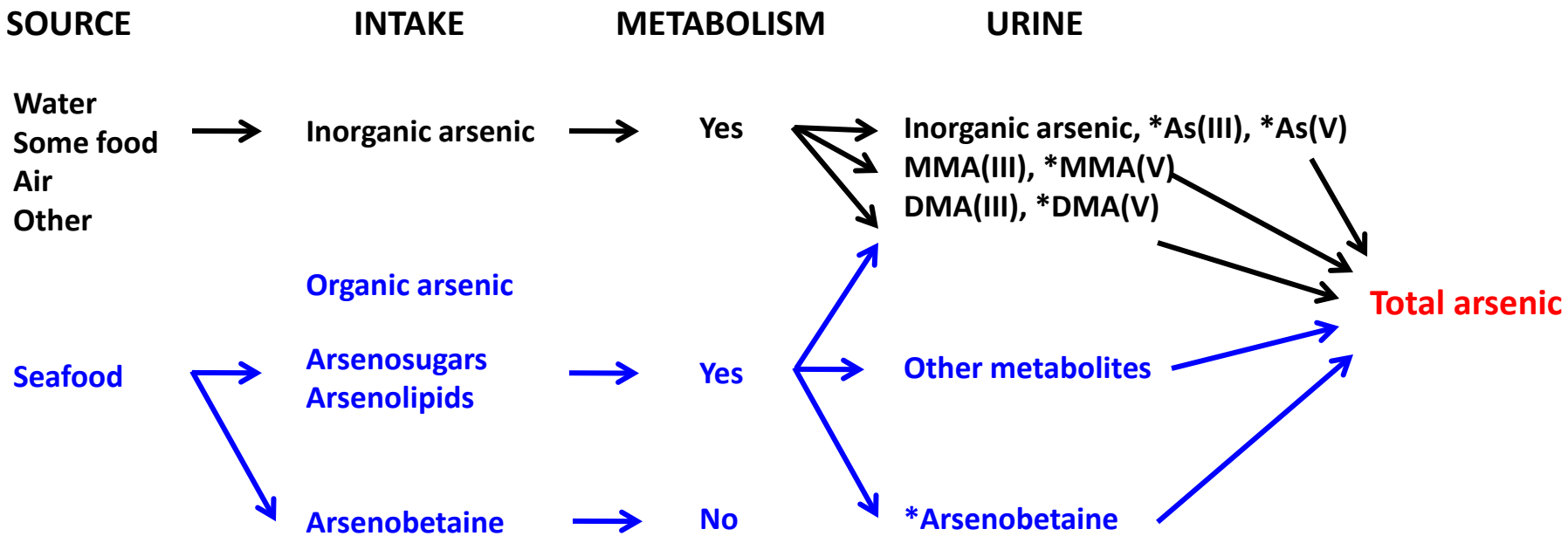
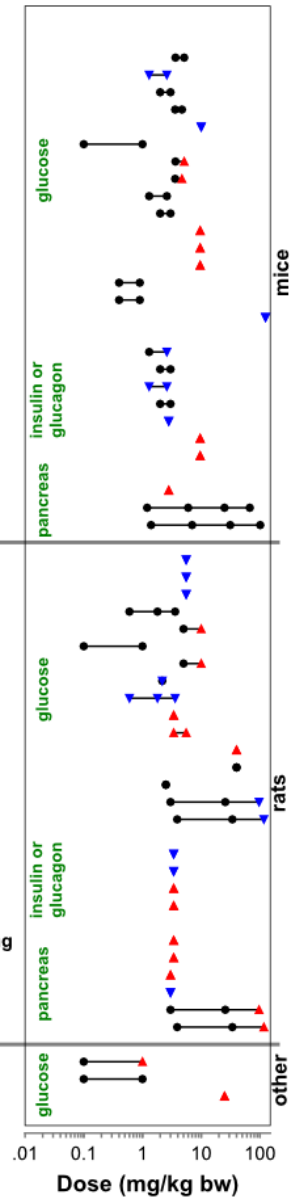


Figure 2. Animal studies of arsenic and endpoints related to glucose homeostasis

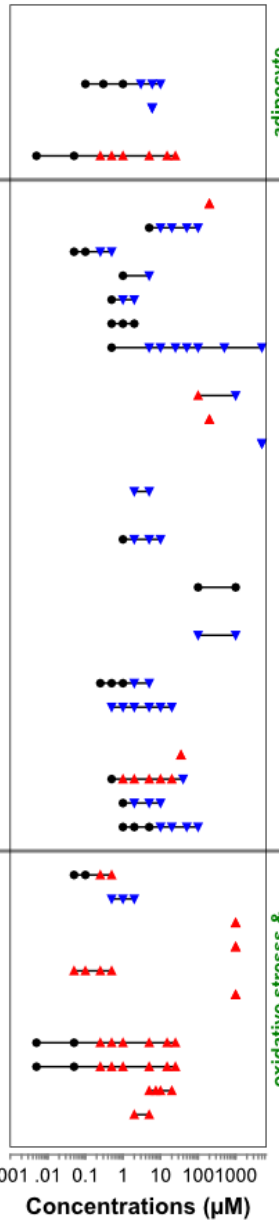
Reference	Chemical	Species, Strain (n, sex)	Route	Treatment Period (lifestage)	Doses (mg/kg bw)	Endpoint
Paul et al. 2007b	As(III)	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[3.65, 5.13]	glucose, blood (fasted)
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[1.3*, 2.6*] + HFD	glucose, blood (fasted)
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[2, 3] + LFD	glucose, blood (fasted)
Paul et al. 2008	As(III)	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[3.6, 4.7]	glucose, blood (fasted)
Boquist et al. 1988	As(III)	mouse, C57BL (5-10 ♂♀)	ip injection	1X (adult)	10*	glucose, blood (fasting NR)
Mitchell et al. 2000	As(III)	mouse, B6C3F1 (3 ♂)	ip injection	1X (adult)	0.1, 1	glucose, blood (non-fasted)
Paul et al. 2007b	As(III)	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[3.65, 5.13*]	glucose, GTT(ip), 2hr
Paul et al. 2008	As(III)	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[3.6, 4.7*]	glucose, GTT(ip), 2hr
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[1.3, 2.6] + HFD	glucose, GTT(oral), 2hr
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[2, 3] + LFD	glucose, GTT(oral), 2hr
Hill et al. 2009	As(V)	mouse, LM/Bc/Fnn (6 ♀)	ip injection	GD 7.5, 8.5 (adult)	9.6*	glucose, GTT(ip), 2hr
Hill et al. 2009	As(V)	mouse, LM/Bc/Fnn (6 ♀)	ip injection	GD 7.5, 8.5 (adult)	9.6*	glucose, plasma (fasted)
Hill et al. 2009	As(V)	mouse, LM/Bc/Fnn (10 ♀)	ip injection	GD 7.5, 8.5 (adult)	9.6*	glucose, plasma (non-fasted)
Paul et al. 2008	MAs(III) oxide	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[0.4, 0.9]	glucose, blood (fasted)
Paul et al. 2008	MAs(III) oxide	mouse, C57BL/6 (5 ♂)	drinking water	8wk (adult)	[0.4, 0.9]	glucose, GTT(ip), 2hr
Judd 1979	MSMA	mouse, P. leucopus (9-10 [♂♀])	drinking water	30d (adult)	[124.5*]	glucose, blood (fasting NR)
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[1.3, 2.6*] + HFD	HOMA-IR
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[2, 3] + LFD	HOMA-IR
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[1.3*, 2.6*] + HFD	insulin, serum (fasted)
Paul et al. 2011	As(III)	mouse, C57BL/6 (9-10 ♂)	drinking water	8wk (adult)	[2, 3] + LFD	insulin, serum (fasted)
Yen et al. 2007	As(III) oxide	mouse, CD-1 ICR (14 ♂)	drinking water	12wk (adult)	[2.8*]	insulin, plasma (fasting NR)
Hill et al. 2009	As(V)	mouse, LM/Bc/Fnn (6 ♀)	ip injection	GD 7.5, 8.5 (adult)	9.6*	HOMA-IR
Hill et al. 2009	As(V)	mouse, LM/Bc/Fnn (6 ♀)	ip injection	GD 7.5, 8.5 (adult)	9.6*	insulin, plasma (fasted)
Yen et al. 2007	As(III) oxide	mouse, CD-1 (ICR) (14 ♂)	drinking water	12wk (adult)	[2.8*]	pancreatitis
Arnold et al. 2003	MMA	mouse, B6C3F1 (52 ♂)	diet	2y (adult)	[1.2, 6.24.9, 67.1]	islet cell adenomas/carcinoma
Arnold et al. 2003	MMA	mouse, B6C3F1 (52 ♀)	diet	2y (adult)	[1.4, 7.31.2, 101.1]	islet cell adenomas/carcinoma
Pal and Chatterjee 2004a	As(III)	rat, Wistar (6 ♂)	ip injection	21d (adult)	5.55*	glucose, blood (fasted)
Pal and Chatterjee 2004b	As(III)	rat, Wistar (6 ♂)	ip injection	30d (adult)	5.55*	glucose, blood (fasted)
Pal and Chatterjee 2005	As(III)	rat, Wistar (6 ♂)	ip injection	30d (adult)	5.55*	glucose, blood (fasted)
Wang et al. 2009	As(III)	rat, SD (5-8 ♂)	drinking water	140-210d (adult)	[0.6, 1.8, 3.6]	glucose, blood (fasting NR)
Ghafghazi et al. 1980	As(III)	rat, CD (4 ♂)	ip injection	7d (adult)	5, 10*	glucose, blood (non-fasted)
Mitchell et al. 2000	As(III)	rat, SD (3 ♂)	ip injection	1X (adult)	0.1, 1	glucose, blood (non-fasted)
Ghafghazi et al. 1980	As(III)	rat, CD (4 ♂)	ip injection	7d (adult)	5, 10*	glucose, GTT(ip), 1hr
Cobo and Castineira 1997	As(III)	rat, Wistar (7 ♂)	drinking water	8wk (adult)	[0.57-2.17*]	glucose, GTT(oral), 2.5hr
Wang et al. 2009	As(III)	rat, SD (5-8 ♂)	drinking water	182d (adult)	[0.6*, 1.8*, 3.6*]	glucose, GTT(oral), 2.5hr
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	glucose, serum (fasted)
Pal and Chatterjee 2005	As(III)	rat, Wistar (6 ♂)	ip injection	30d (adult)	5.55*	glucose, urine (fasted)
Singh and Rana 2009	As(III) oxide	rat, Wistar (5 ♂)	ip injection	30d (adult)	40*	glucose, serum (fasted)
Singh and Rana 2009	As(III) oxide	rat, Wistar (5 ♂)	ip injection	30d (adult)	40	insulin, serum (fasted)
Aguilar et al. 1997	As(V) oxide	rat, Wistar (5 ♂)	diet	10wk (adult)	[2.5]	glucose, blood (non-fasted)
Arnold et al. 2003	MMA	rat, F344 (60 ♂)	diet	2y (adult)	[3, 25.7, 65.8-96.7*]‡	glucose, blood (fasted)
Arnold et al. 2003	MMA	rat, F344 (60 ♀)	diet	2y (adult)	[3.9, 33.9, 67.9-116.8*]‡	glucose, blood (fasted)
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	glucagon, serum ([fasted])
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	glucose:insulin ratio (fasted)
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	HOMA-IR
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	insulin, blood (fasting)
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	altered insulin/glucagon staining
Izquierdo-Vega et al. 2006	As(III)	rat, Wistar (10 ♂)	oral (gavage)	90d (adult)	3.4*	oxidative stress
Mukherjee et al. 2006	As(III)	rat, Albino (5 ♂)	oral (not specified)	30d (adult)	3*	oxidative stress
Mukherjee et al. 2006	As(III)	rat, Albino (5 ♂)	oral (not specified)	30d (adult)	3*	pancreatic islet cells
Arnold et al. 2003	MMA	rat, F344 (60 ♂)	diet	2y (adult)	[3, 25.7, 65.8-96.7]‡	pancreatitis
Arnold et al. 2003	MMA	rat, F344 (60 ♀)	diet	2y (adult)	[3.9, 33.9, 67.9-116.8]‡	pancreatitis
Mitchell et al. 2000	As(III)	guinea pig, Hartley (3 ♂)	ip injection	1X (adult)	0.1, 1*	glucose, blood (non-fasted)
Mitchell et al. 2000	As(III)	hamster, Golden-Syrian (3 ♂)	ip injection	1X (adult)	0.1, 1	glucose, blood (non-fasted)
Biswas et al. 2000	As(III)	goat, Black Bengal (6 ♀)	oral (capsule)	12wk (adult)	25*	glucose, blood (non-fasted)



● no statistically significant effect reported
▲ statistically significant increase reported
▼ statistically significant decrease reported

Figure 3. In vitro studies related to arsenic and diabetes

Reference	Chemical	Cell Type, Species	Concentrations (μM)	Endpoint
Trouba et al. 2000	As(III)	C3H 10T1/2 pre-adipocytes, mouse	0.1, 0.3, 1, 3*, 6*, 10*	adipocyte differentiation
Wauson et al. 2002	As(III)	C3H 10T1/2 pre-adipocytes, mouse	6*	aP2, PPARg, C/EBPa mRNA levels
Salazard et al. 2004	As2O3	3T3-F442A pre-adipocytes, mouse	0.005, 0.05, 0.25*, 0.5*, 1*, 5*, 15*, 25*	PPARg, C/EBPa expression
Widnell et al. 1990	As(III)	BHK cells, hamster	200*Δ	glucose transporter (altered distribution)
Walton et al. 2004	As(III)	3T3-L1 adipocytes, mouse	5, 10*, 20*, 50*Δ, 100*Δ	glucose uptake
Fu et al. 2010	As(III)	INS-1 (832/13) β cells, rat	0.05, 0.1, 0.25*, 0.5*	GSIS
Diaz-Villasenor et al. 2006	As(III)	isolated β cells, rat	1, 5*	GSIS
Diaz-Villasenor et al. 2008	As(III)	RINm5F β cells, rat	0.5, 1*, 2*	GSIS
Diaz-Villasenor et al. 2008	As(III)	RINm5F β cells, rat	0.5, 1, 2	insulin synthesis
Paul et al. 2007a	As(III)	3T3-L1 adipocytes, mouse	0.5, 5*, 10*, 25*, 50*, 100*, 500*, 5000*	insulin-stimulated glucose uptake
Walton et al. 2004	As(V)	3T3-L1 adipocytes, mouse	100*, 1000*	glucose uptake
Quintanilla et al. 2000	As(V)	liver Clone 9 cells, rat	200*Δ	glucose uptake
Orsater et al. 2002	As(V)	ob/ob, isolated islets, mouse	5000*Δ	GSIS
Lu et al. 2011	As2O3	RINm5F β cells, rat	2*, 5*	insulin secretion
Walton et al. 2004	DMA(III) oxide	3T3-L1 adipocytes, mouse	1, 2*, 5*Δ, 10*Δ	glucose uptake
Walton et al. 2004	DMA(V)	3T3-L1 adipocytes, mouse	100, 1000	glucose uptake
Walton et al. 2004	MAs (V)	3T3-L1 adipocytes, mouse	100*, 1000*	glucose uptake
Walton et al. 2004	MAs(III) oxide	3T3-L1 adipocytes, mouse	0.25, 0.5, 1, 2*Δ, 5*Δ	glucose uptake
Paul et al. 2007a	MAs(III) oxide	3T3-L1 adipocytes, mouse	0.5*, 1*, 2*, 5*, 10*, 20*	insulin-stimulated glucose uptake
Widnell et al. 1990	PhAsO	BHK cells, hamster	35*Δ	glucose transporter (altered distribution)
Scott et al. 2009	PhAsO	L929 fibroblasts, mouse	0.5, 1, 2*, 5*, 10*, 20*, 30*, 40*	glucose uptake
Liebl et al. 1995a, 1995c	PhAsO	MDCK cells, dog	1, 2*, 5*, 10*	glucose uptake
Liebl et al. 1995b	PhAsO	MDCK cells, dog	1, 2, 5, 10*, 20*, 50*, 100*	glucose uptake
Fu et al. 2010	As(III)	INS-1 (832/13) β cells, rat	0.05, 0.1, 0.25*, 0.5*	cellular oxidative stress
Diaz-Villasenor et al. 2008	As(III)	RINm5F β cells, rat	0.5*, 1*, 2*	intracellular [Ca2+]
Macfarlane et al. 1997	As(III)	isolated islet cells, human	1000*Δ	IUF1 binding to DNA
Macfarlane et al. 1997	As(III)	MIN6 β cells, mouse	1000*Δ	MAPKAP-K2 activity
Fu et al. 2010	As(III)	INS-1 (832/13) β cells, rat	0.05*, 0.1*, 0.25*, 0.5*	Nrf2 protein in nucleus
Macfarlane et al. 1999	As(III)	isolated islet cells, human	1000*Δ	PDX1 activation & translocation to nucleus
Salazard et al. 2004	As2O3	3T3-F442A pre-adipocytes, mouse	0.005, 0.05, 0.25*, 0.5*, 1*, 5*, 15*, 25*	cell-cycle c-jun, KLF5 expression
Salazard et al. 2004	As2O3	3T3-F442A pre-adipocytes, mouse	0.005, 0.05, 0.25*, 0.5*, 1*, 5*, 15*, 25*	HO1, HIF1a expression
Yen et al. 2007	As2O3	HIT-T15 β-cells, hamster	5*, 7.5*, 10, 20*	ROS
Lu et al. 2011	As2O3	RINm5F β cells, rat	2*, 5*	ROS



● no statistically significant effect reported
▲ statistically significant increase reported
▼ statistically significant decrease reported